Simultaneous multi-cancer detection and tissue of origin (TOO) localization using targeted bisulfite sequencing of plasma cell-free DNA (cfDNA)

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Disclosures

The Circulating Cell-free Genome Atlas (CCGA) Study: Supporting Development of a Multi-Cancer Test

Prospective, observational, longitudinal, case-control study for the discovery, training, and validation of a multi-cancer test

15,254 participants
with & without cancer

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142 sites

Fully Enrolled

Blood samples
(from all participants)

Tissue samples
(cancer only)

Follow-up for 5 years
(vital status & cancer status)

Targeted Methylation Assay for Further Development

Pre-specified studies for discovery and validation

3 pre-specified CCGA sub-studies

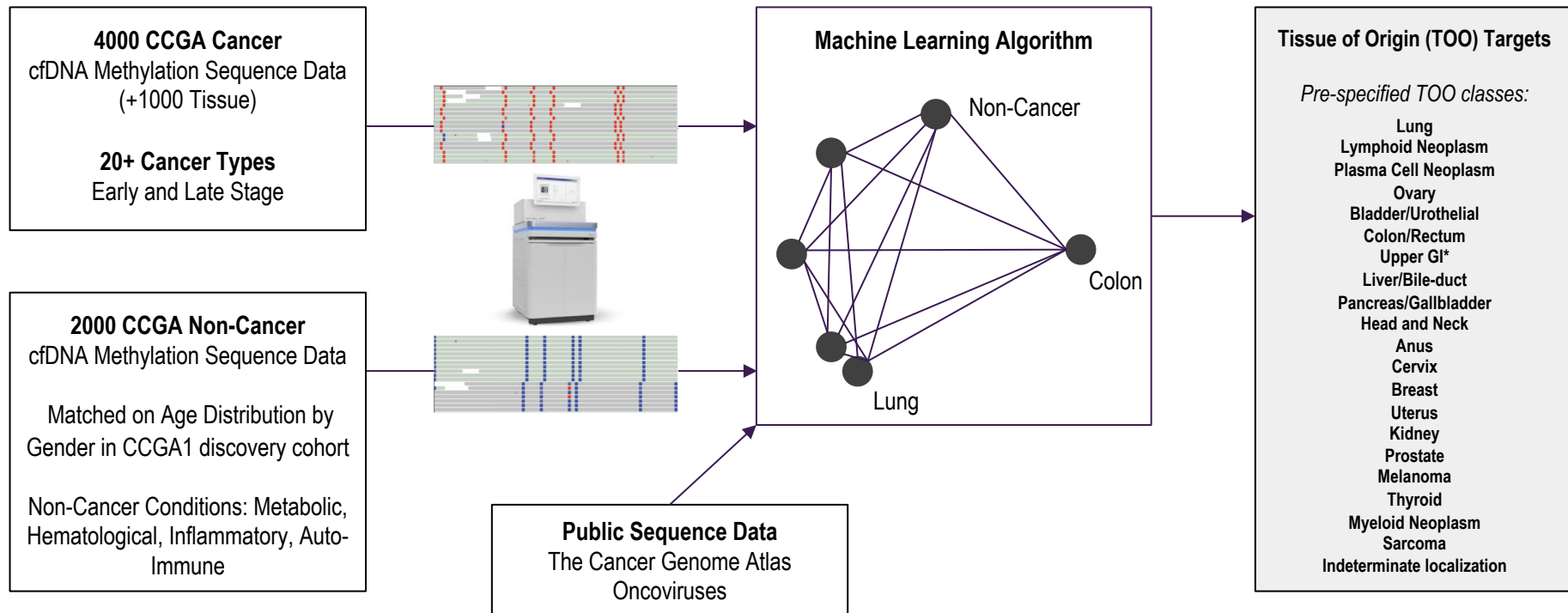| **1. Discovery** 2,800 participants | **2. Training/ Validation** ~4,500 participants | **3. Further Validation** ~5,000 participants |
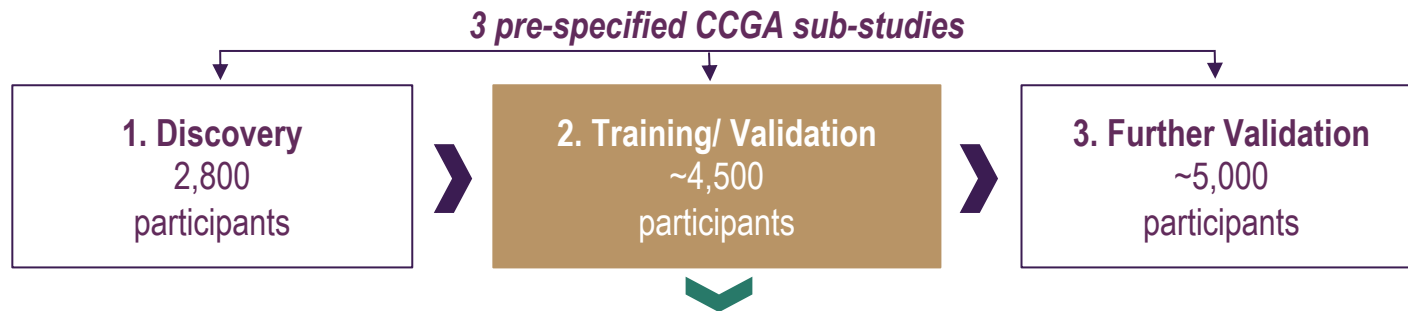
Discovery phase completed in 2018[1,2]

Targeted methylation was selected for further assay development, including training and internal cross-validation[3]

Here, we report on an independent validation set that is ongoing

1. Klein EA, *et al. J Clin Oncol* 2018;36(15_suppl):12021. 2. Liu MC, *et al. J Clin Oncol* 2018;36(15_suppl):536. 3. Oxnard GR, *et al*. ESMO 2019; Barcelona, Spain. Abstract 5639.

Target Selection Using Machine Learning Algorithm

Targeted methylation panel developed through generation and analysis of an extensive database of plasma and tissue methylation patterns

4000 CCGA Cancer
cfDNA Methylation Sequence Data
(+1000 Tissue)

20+ Cancer Types
Early and Late Stage

2000 CCGA Non-Cancer
cfDNA Methylation Sequence Data

Matched on Age Distribution by Gender in CCGA1 discovery cohort

Non-Cancer Conditions: Metabolic, Hematological, Inflammatory, Auto-Immune

Public Sequence Data
The Cancer Genome Atlas
Oncoviruses

Machine Learning Algorithm

Non-Cancer

Colon

Lung

Tissue of Origin (TOO) Targets

Pre-specified TOO classes:

Lung
Lymphoid Neoplasm
Plasma Cell Neoplasm
Ovary
Bladder/Urothelial
Colon/Rectum
Upper GI*
Liver/Bile-duct
Pancreas/Gallbladder
Head and Neck
Anus
Cervix
Breast
Uterus
Kidney
Prostate
Melanoma
Thyroid
Myeloid Neoplasm
Sarcoma
Indeterminate localization

*Upper GI combines esophageal and gastric cancers: diagnostic workup covers both cancer types.

# Stage-specific Performance was Consistent between Training and Validation Sets

- Cross-validated training set was independently tested in the validation set

- Trained classifier targeted >99% specificity and achieved a consistent <1% FPR in the training and validation sets (P=0.095)

- Training and validation set detection rates were consistent across stages for the pre-specified cancers and all cancers

- Confirms training data were not overfitted

More than 20 Cancer types Detected at Early and Late Stages

At 99.3% specificity:
- Sensitivity overall was 55% (51-59%)
- Sensitivity in pre-specified† cancer types was 76% (72-81%)

A. All Cancers* (>20 Types)   B. Pre-specified Cancers†
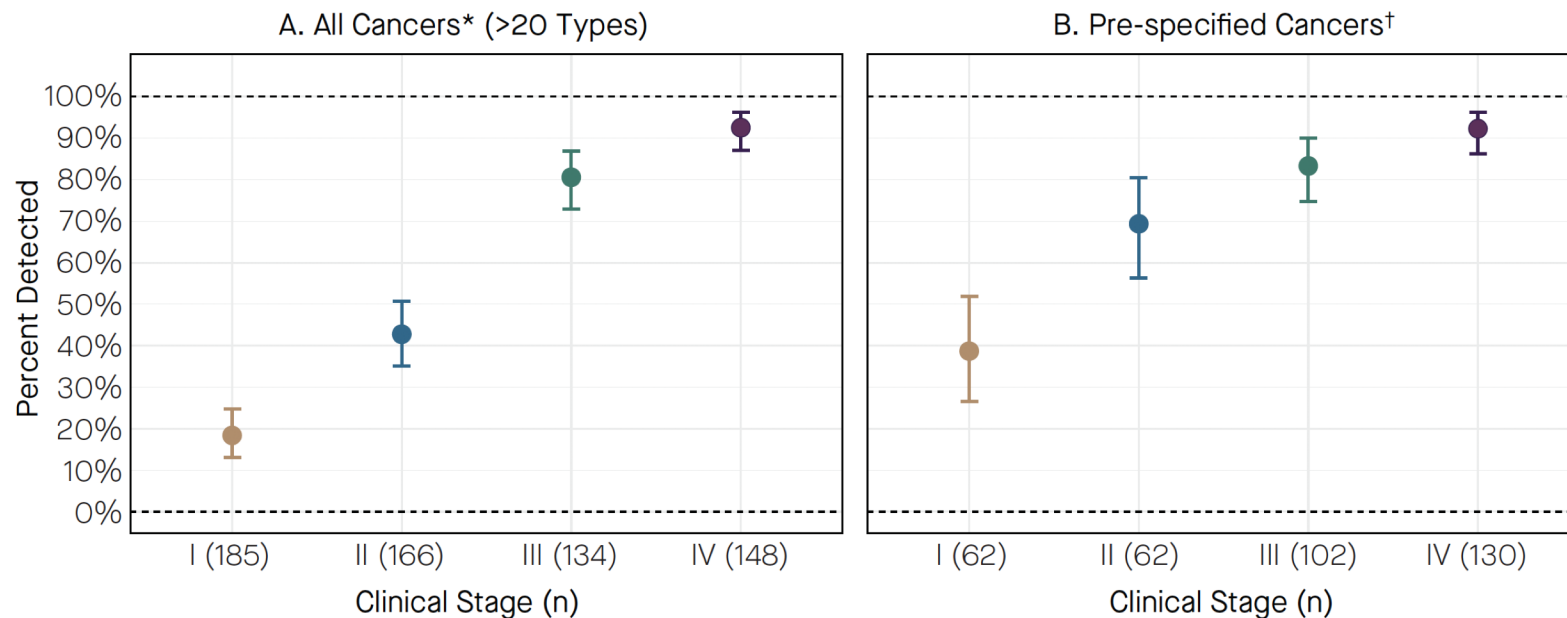
*Plot excludes unstaged cancers. †Includes anal, bladder, colorectal, esophageal, head and neck, liver/bile-duct, lung, lymphoma, ovary, pancreatic, plasma cell neoplasm, stomach cancer..

Highly Accurate Tissue of Origin (TOO) Localization

- 96% of samples with assigned TOO

- 93% of those calls were correct

- Highly precise localization to a single tissue site across >20 distinct tumor types
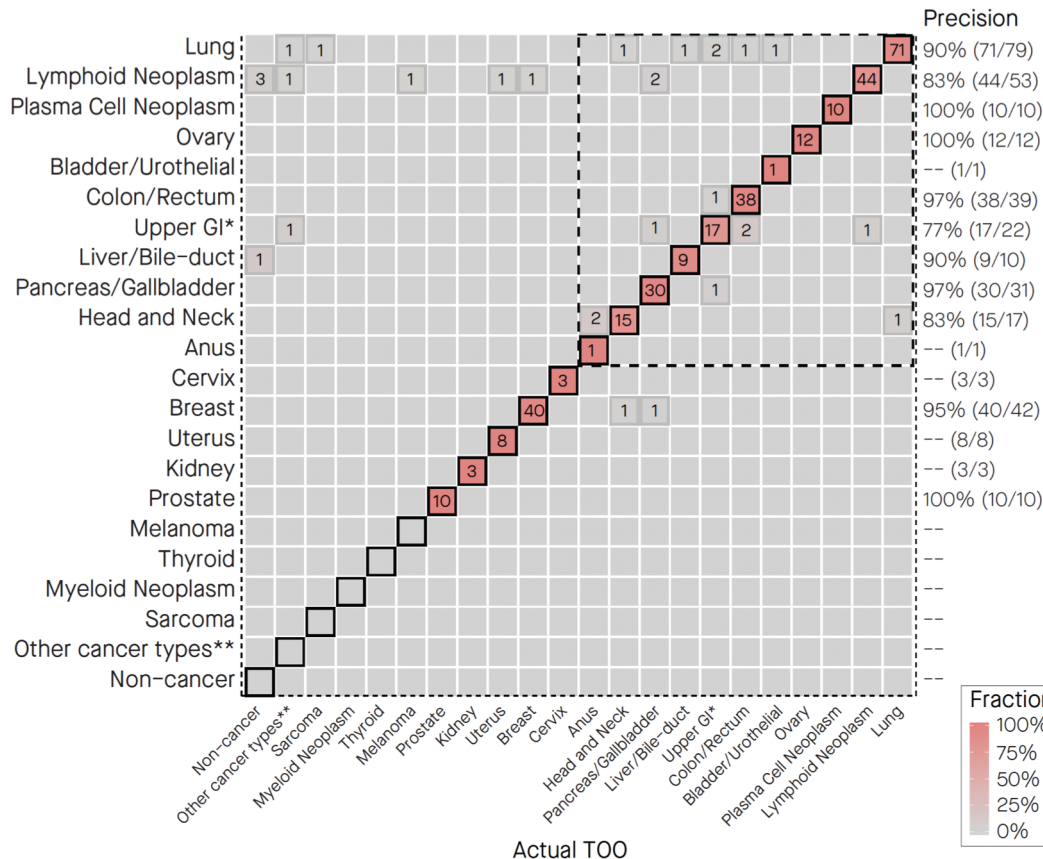

*Upper GI combines esophageal and gastric cancers: diagnostic workup covers both cancer types.

**Other cancer types= skin cancer (not including basal cell carcinoma, squamous cell carcinoma, or melanoma), testis, seminoma, vagina, and vulva.

Tissue of Origin (TOO) Accuracy is Consistently High Across Stages

- Single tissue localization: **93% of TOO calls were correct**
- Localization to the top two TOO calls: **95% of TOO calls were correct**

A. All Cancers* (>20 Types)

B. Pre-specified Cancers†
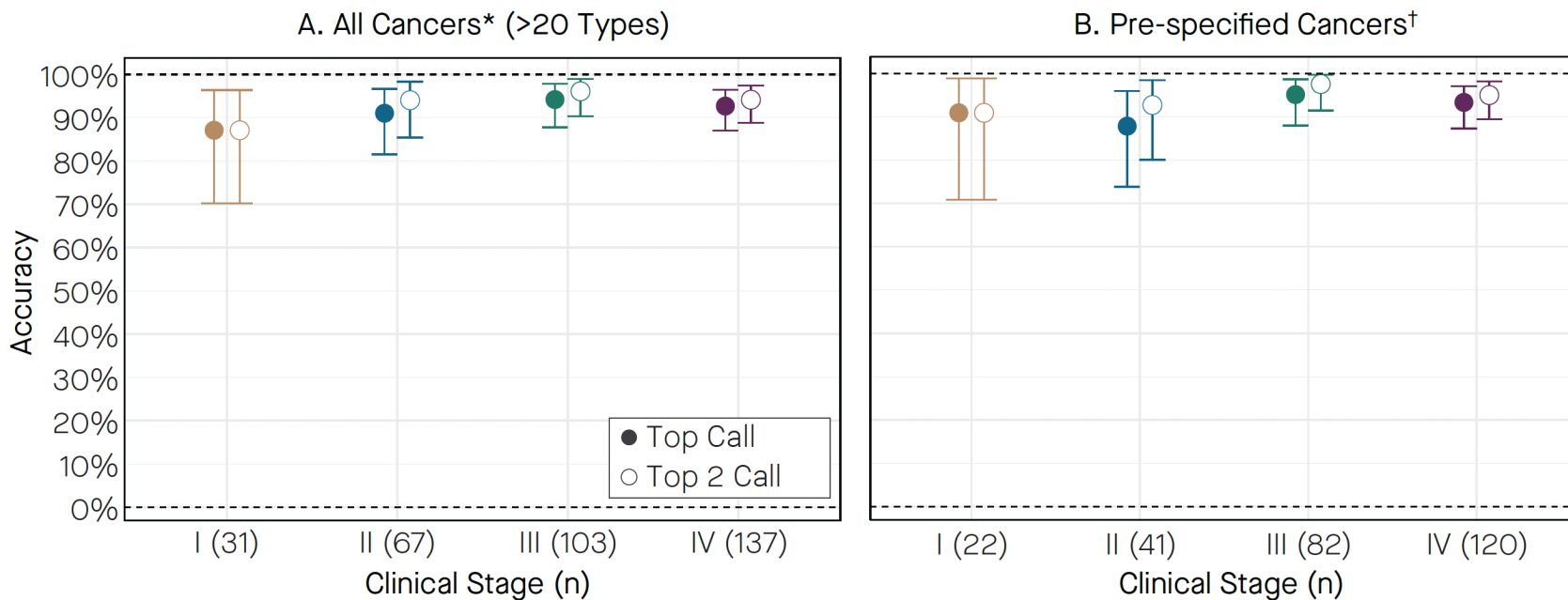
*Lymphoid leukemias, myeloid neoplasms, and brain. †Includes anal, bladder, colorectal, esophageal, head and neck, liver/bile-duct, lung, lymphoma, ovary, pancreatic, plasma cell neoplasm, stomach.

Conclusions

- Multiple deadly cancer types that currently have no screening paradigm were detected across stages and simultaneously accurately localized to a TOO using methylation signatures in plasma cfDNA.

- This was achieved with trained thresholds that resulted in a single, fixed, low false positive rate (<1%) in an independent validation set.

- Importantly, results in the independent validation set were indistinguishable from the training set, demonstrating the robustness of machine learning classifier training, with no evidence of overtraining.

- This validation demonstrates feasibility of a single blood-based test that can simultaneously detect multiple cancers and supports further clinical development to prepare for the return of results.

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